BIODEGRADATION OF CELLULOSE IN THE GUT OF HEPTACARUS HIRSUTUS WALLWORK, 1964 (ACARI, ORIBATEI)

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БИОЛОГИЧЕСКОЕ РАЗРУШЕНИЕ ЦЕЛЛЮЛОЗЫ В КИШЕЧНИКЕ HEPTACARUS HIRSUTUS WALLWORK, 1964 (ACARI, ORIBATEI)

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Abstract

Heptacarus hirsutus, collected from the Beypore beach was studied with respect to its gut microflora. It was found that microbial involvement was higher in the tritonymph and adult as compared to the other immature stages. The flora were almost all bacterial. The most abundant bacteria isolated from the tritonymph and adult were proved to be cellulolytic using weight loss of filter paper strips as the criteria. The weight loss appeared significant when analysed statistically.

Introduction

Recent awareness of the importance of oribatid mites in the decomposition of plant residues has thrown several new and interesting informations on their feeding habits and digestive capabilities. As a result, several species are now known capable of decomposing the complex plant materials consumed by them. While stressing the importance of oribatid mites /9/ the cited author insisted that the real value of oribatids in decomposition can be ascertained only through a disclosure of the mechanism by which they are able to break down cellulose, lignin and other components of the wood. Litter disappearance in the tropical forest at Ibadan was influenced by oribatid mites /11/. There is the opinion /3/ that in Belgium woodland 20% of the annual leaf fall was passed through the gut of macrophytophagous oribatid. Results of the enzyme assays /10/ on fourteen species of oribatid mite from Danish beech wood soil established the presence of many enzymes responsible for digestion of complex polysaccharides. Study on the feeding activity of a phthiracarid mite /5/ helped much in elucidating its functional significance to the solid type of food which is ingested. Discussing the digestive ability of the above mite the cited author expressed the opinion that possession of cellulase need not necessarily enable the mites to digest cell wall as this constitutes a magnitude of substances. A knowledge on the feeding habits of oribatid mites is essential for the analysis of their role as promoters of soil fertility /1/. It was suggested /7/ the possibility of gut symbionts to have adapted themselves to food materials and synthesize specific enzymes. Fungal colonies from Phthiracarus sp. and Steganacarus magnus which were previously considered to be macrophytophages were detected /2/. Feeding

activity of Heptacarus hirsutus on wood /8/ was influenced by its possession of an ideal microbial community.

Materials and Methods

The plant debris accumulated during low tides in two sandy mounts heavily deposited with bivalve shells /8/ comprises the collection site. Frequent samples were obtained between 6 and 7 a.m. mostly from the upper few centimeters of the soil, with the help of a shovel. Collected samples were subjected to extraction in a series of modified Tullgren funnels for a period of not less than 72 hours.

Mites were extracted live in powdered plant debris previously moistened and kept in a test tube at the tail end of the funnel. After extraction the tubes were removed and a small portion of the contents was thinly spread in a petridish. It was then dried under a table lamp of 100 w for 5 to 10 minutes. When the mites started moving, the adults and nymphs were picked up separately with a fine camel hair brush and stored in tubes (5 cm height). The mites were then surface sterilized by thoroughly washing them in 1% chlorine solution for 1-3 minutes and then in distilled water respectively. The cleaned mites were introduced in batches of 5-10 into specially prepared and sterilized culture chambers. A culture assembly consisted of 5 glass rings closed with cover slips spaced out on filter paper, arranged on a glass plate. Water introduced on to the filter paper provided the required humidity in the glass ring where the mites have been previously kept. Several such assemblies were maintained at a time. Routine examination of the mites was carried out by using a stereomicroscope (30x magnification) in a contaminant free glass chamber.

When faecal pellets were passed, they were aseptically picked up with a moist needle and suspended in known quantities of distilled water. After appropriate dilutions, aliquots of this solution were placed in petridishes of 8 x 5 cm diameter. To detect bacteria and fungi nutrient agar and PDA were poured in respectively, mixed and incubated after solidification. Control plates were also maintained to rule out contamination of media. When colonies developed, they were counted, isolated and subcultured in fresh plates to obtain pure cultures. These were then identified.

The cellulose feeding activity of the adult and nymphs was tested by providing them filter paper bits. Digestive activity of the nymphal stages was mainly focussed on, as here the microbes were in abundance. Previously weighed filter paper bits 5 to 6 cm long and 1 cm wide after inoculating with microbes were introduced into test tubes containing 5 ml of modified Czapek broth each and incubated till sufficient growth showed up. The undigested filter material was taken out, the microbial deposit carefully removed with a soft brush, dried and weighed. Percentage loss in weight of the filter material was worked out. A statistical analysis of the data involving t-test was conducted to find out the significance of loss of weight.

Observation and Discussion

Further to the previous report on H.hirsutus /8/, in the present investigation the nymphal stages of the animals were also subjected to microbial analysis of faeces in addition to the adult. Table 1 shows that the number

T a b l e 1. Incidence of microbial types in the gut of nymphs and adults of Heptacarus hirsutus

	Gram Nature	Morphology	Protonymph	Deutonymph	Tritonymph	Adult
Bacteria	-	Rod	-	+ +	+ +	+
	-	Rod	+	+ +	+ + +	+
	-	Rod	+ +	+	+ + +	+ +
	-	Rod	+ +	+ +	+ + +	+
	-	Rod	+ +	+	+ + +	+ +
	-	Coccobacilli	+ +	+	+ +	+
Actino-Mycetes	+		+	+	+ +	+ +

N o t e s: (-)-Absent; (+)-Present; (++)-Abundant; (+ + +)-More Abundant.

and nature of bacterial colonies obtained from the faeces of the different growth stages of the above mite varied considerably. Bacterial colonies showed up after 24 hours of incubation and increased in number with time. This increase was all the more specific in nymphal plates particularly with that of tritonymph, in which the number of bacterial colonies was about twice as that of the adult on the fourth day. These bacteria were gram negative short rods and coccobacilli. Gram positive Actinomycetes occurred both in adult and nymphal plates but their number was more in tritonymph and adult. Fungal colonies were absent in adult and juvenile plates except in one case where a Fusarium sp. colony was noted in the adult plate and ruled out as a contamination.

The experiments on faecal analysis of the adult and nymph revealed that under natural condition the gut of this mite harbours a variety of microbial communities. Such microbial communities have been reported in the alimentary canal of Steganacarus diaphanum, Achipteria coleoptrata and Oppia nitens /9, 13/ while assessing their role in the decomposition of plant materials. Wide varieties of microbial population are available to these mites in nature but only a particular stage in the lift history of this mite is found to harbour high proportions of these. A qualitative and quantitative difference is usually noted between faecal matter and the ingested food /4,15/. Such a modification of the ingested food predisposes the faecal matter to microbial attack /2/. The faeces of tritonymph that yielded the maximum number of bacterial colonies, may be considered more significant in decomposition process compared to other stages. A proportionate increase in the microbial communities in voraciously feeding immature stages may be taken as a pre-requisite for leading such an active role in plant disintegration. This may be one of the reasons for the tritonymph being richer with respect to bacterial abundance. Presence of microbial encrustrations on the food /9,14,8/ may,no doubt, afford a conditioning of it to make it acceptable to oribatids, particularly by the macrophytophages, due to better physico-chemical climate in the gut to help the mite significantly in its digestive function. Thus, though the digestion of the recalcitrant food inside the gut may be a major

Table 2. Quantitative analysis of cellulolytic degradation of adults and tritonymphs Heptacarus hirsutus

Stages	Initial weight, mg	Final weight, mg	Loss of weight, mg	Loss of weight, %	Loss of weight, mean %	Standard deviation	t-value
	33.00	32.04	0.96	2.909			
	37.50	36.01	1.49	3.974			
Adult	30.90	31.82	1.64	5.307	5.3582	2.276	
	30.65	27.17	2.88	9.585			
	30.50	31.82	1.68	5.016			
	32.65	29.38	3.27	10.01			5,637 (P<0,01)
	37.20	34.90	2.30	6.183			
Tritonymph	37.45	35.34	2.11	5.633	7.3636	1.843	
	37.25	35.05	2.20	5.906			
	36.10	32.82	3.28	9.086			

aspect, the role of the microbes in rendering the food acceptable to the mite is crucial.

The faecal cultures conclusively proved the presence of Actinomycetes in all the stages studied, but its further identification is in progress. Abundance of Actinomycetes increased after the deutonymphal stage and carried on into the adult. Investigations made on the microflora of the alimentary canal of A.coleoptrata /12/ clearly showed that Actinomycetes were more abundant and active in juvenile stages than in the adult. This discrepancy can only be explained on the basis of the soil microflora in the original sites of collection of the two mite species studied.

Though incidence of the fungal remants in the adult gut of H.hirsutus has been reported /6/, its scarce consumption of Trichoderma sp. and that too during starvation alone /8/, is the closest demonstration of fungal preference by the mite. However, in A.coleoptrata /12/ five species of fungi could be detected from the adult and juvenile stages collected from field. This may possibly explain a difference in feeding habits exhibited by A.coleoptrata and H.hirsutus. Alternatively, it may well be that the site of collection was very low in fungus due to the highly putrifactive (as against saccharolytic) nature of fungi prevalent there on account of fall out from huge quantity of bivalve shells deposited.

Further to the previous report from this laboratory /8/ an attempt has been made here to quantify the extent of microbial involvement in the adult and nymph of H.hirsutus. Also, the comparison of the most abundant microbe from the gut of adult and nymph with respect to their cellulolytic property, has been made (Table 2). In this regard, the choice of weight loss as an index of microbial growth was initially made for reasons of simplicity. Sufficient growth of microbial colonies on filter strips was noted which increased considerably on successive days of examinations in all the experimental cases. This was apparent from the number of microbial spots developed on the

filter strips. Quantitative assessment of weight loss in filter strips showed that tritonymphs are provided with abundant and more active gut microbes. This indicated an active involvement of the tritonymph in cellulolytic degradation than that of the adult. Statistical analysis of the weight loss in filter paper through t-test showed that it is significant at 1% level.

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